https://doi.org/10.56946/jspae.v4i2.605 ISSN: 2957-9082
Journal of Soil, Plant and Environment

ORIGINAL RESEARCH

Ethidium Bromide-Induced Genetic Variability and Drought Tolerance in Cowpea (*Vigna unguiculata* L. Walp.) Under Field Conditions

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Received: 04 January 2025
Revised: 05 July 2025
Accepted: 29 August 2025

ABSTRACT: Drought stress significantly reduces cowpea yields in Africa, necessitating the development of drought-resilient genotypes. This study evaluated the genetic variability and drought tolerance of nine ethidium bromide (EtBr)-derived cowpea genotypes at the M7 generation under control and drought stress conditions. The study was conducted in a randomized complete block design and assessed morphological and yield traits alongside ten drought tolerance indices (DTIs). Significant effects of genotype and genotype × treatment interactions were observed for most traits, except peduncle length and 100-seed weight. Genotypes G1 and G2 demonstrated superior drought tolerance, reflected by high values for key DTIs such as Geometric Mean Productivity (GMP), Stress Tolerance Index (STI), and Drought Resistance Index (DRI), and consistently maintained higher yields under stress. In contrast, G5 and G7 showed poor performance under drought, with lower yields and DTI values. Broad-sense heritability was high for important traits, including plant height (84.41%) and seed yield per plant (60.08%), indicating strong genetic control. High genotypic and phenotypic coefficients of variation, particularly for seed yield per plant (GCV: 71.54%, PCV: 92.29%), suggest considerable potential for selection-based improvement. The heatmap analysis revealed that reproductive traits, particularly seed yield, number of pods, and peduncle length, are strongly associated with key DTIs, making them valuable targets for selection under drought stress. These findings underscore the effectiveness of EtBr-induced mutagenesis in generating genetic variability and enhancing drought resilience in cowpea. Future breeding programs should prioritize genotypes like G1 and G2, integrating key drought-related traits and indices to develop high-yielding, climate-resilient cowpea varieties suitable for drought-prone regions in sub-Saharan Africa.

KEYWORDS: Cowpea, drought tolerance, drought tolerance indices, ethidium bromide-induced variability, genetic variability.

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1. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an annual herbaceous legume widely cultivated across tropical and subtropical regions, particularly in Sub-Saharan Africa (SSA). It plays a vital role in food security, human nutrition, income generation, and livestock feeding systems. Nutritionally, cowpea grains contain high levels of protein (23–32%), carbohydrates, folic acid, and essential minerals (Carvalho et al., 2017), while the leaves offer an additional protein source (27–34%) (Owusu et al., 2021). Its seeds serve as a primary dietary protein

source and are traditionally used to support digestive health, including eliminating intestinal parasites (Alfa et al., 2020).

Beyond its nutritional attributes, cowpea exhibits numerous health-promoting properties. Studies have shown its potential in preventing diabetes, lowering blood lipids and blood pressure, reducing inflammation, and combating cancer. These benefits are attributed to its content of dietary fiber (both soluble and insoluble), phytochemicals, proteins, and peptides, which contribute to the prevention of chronic diseases (Chathuni et al., 2018). For resource-poor populations whose diets are often based on starchy staples such as maize, sorghum, millet, and cassava, cowpea represents a valuable means of addressing protein-energy malnutrition (Ddungu et al., 2015; Horn et al., 2022).

Agronomically, cowpea provides several advantages. It is a fast-growing crop capable of providing ground cover to reduce erosion, and it contributes to soil fertility through biological nitrogen fixation. It also requires minimal external inputs and thrives across a wide pH range compared to other legumes (Ajayi et al., 2018; Abdou-Razakou et al., 2013). Despite its adaptability and agronomic potential, cowpea productivity remains constrained by poor agronomic practices, limited input use, and the cultivation of unimproved varieties (Horn et al., 2022).

Cowpea grows optimally in environments receiving 500–1200 mm of annual rainfall and average temperatures between 28 and 30°C during the growing period (Craufurd et al., 1996; Owusu et al., 2021). Globally, cowpea is cultivated on approximately 14.5 million hectares, yielding around 6.5 million tons annually, with Africa contributing over 83% of total production (Boukar et al., 2018; Kebede and Bekeko, 2020). Nigeria is the leading producer, accounting for 61% of Africa's and 58% of global cowpea production (Nkomo et al., 2021).

However, average yields across SSA remain low—often below 600 kg/ha—far below the crop's yield potential of over 2000 kg/ha. This yield gap is attributed to biotic and abiotic stresses such as pests, diseases, parasitic weeds, poor soil fertility, and, most notably, drought (Rugare et al., 2013; Boukar et al., 2016; Huynh et al., 2016; Bolarinwa et al., 2022). Drought stress poses a grave challenge, limiting yield by disrupting physiological processes such as stomatal conductance, evapotranspiration, and photosynthesis, ultimately stunting plant growth (Gomes et al., 2020). With increasing climate variability, the development and adoption of drought-resilient cowpea genotypes are essential to achieving food security in drought-prone areas (Fatokun et al., 2018).

Traditional breeding approaches have had limited success due to cowpea's self-pollinating nature, which reduces genetic variability (Eswaramoorthy et al., 2021). To overcome this limitation, a systematic breeding strategy that enhances genetic variation is needed. Mutation breeding is a promising tool in this regard. By applying physical or chemical mutagens, novel genetic variation can be introduced into self-pollinated crops, enabling improvements in yield, seed quality, and stress tolerance (Ajayi et al., 2010). Induced mutagenesis has proven successful in improving agronomic traits in numerous crops (Naik and Murthy, 2009), and it holds significant potential for

enhancing cowpea productivity. Mutation breeding using chemical mutagens such as ethidium bromide (EtBr) is a promising strategy for enhancing genetic variability in self-pollinated crops. As a potent mutagen, EtBr induces mutations in mitochondrial, chloroplast, and nuclear DNA, generating novel phenotypes and traits that are not easily achieved through conventional breeding. This approach can improve yield, seed quality, and stress tolerance by introducing valuable genetic diversity (Stephens, 2009; Keadtidumrongkul et al., 2018).

Therefore, this study aims to evaluate the genetic variability and determine character associations among ethidium bromide (EtBr)-induced cowpea genotypes subjected to early-stage drought stress under M7 generation conditions. The specific objectives are to assess genetic variability among EtBr-induced cowpea genotypes at the M7 generation under early drought stress by evaluating morphological and yield-related traits in both stress and non-stress conditions. The study seeks to estimate drought tolerance indices (DTIs) and compute genetic parameters such as heritability, genetic advance, and coefficients of variation, which reflect the potential for genetic improvement of yield and drought-related traits. Furthermore, it analyzes character associations to identify significant trait correlations and select high-performing, drought-tolerant genotypes suitable for subsequent breeding and improvement programs.

2. Materials and methods

2.1 Source of plant materials

This study utilized nine cowpea genotypes derived from accession ITK98K-555-1 by treatment with 0.2% and 0.5% (v/v) ethidium bromide at different durations and subsequently raised to M6 (see Table 1). These genotypes were obtained from the Plant Breeding Unit, Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria.

2.2 Experimental site details

The research commenced during the dry season from January 19th to the rainy season on July 8th, 2022, in the Department of Plant Science and Biotechnology research plot, Adekunle Ajasin University, Akungba-Akoko. The field is situated at Latitude 7.2°N, Longitude 5.44°E, and an altitude of 423 meters above sea level in Ondo State, Nigeria. The study site is located in southwestern Nigeria and experiences a tropical climate characterized by distinct rainy and dry seasons. The rainy season typically extends from March to November, with a brief dry period in August known as the "August break." The region receives an average annual rainfall of approximately 1,150 mm, ranging between 800 mm and 1,500 mm, with about 85% of the total rainfall concentrated between June and September. The average air temperature is 24.7°C, while the annual relative humidity ranges from 41% to 91%. The soil at the site is classified as sandy loam ultisol. Baseline physico-chemical properties of the experimental soil were previously assessed, as reported by Ajayi et al. (2020). Initial soil characteristics included a pH of 6.50, total organic matter (TOM) content of 2.60%, total nitrogen (TN) of 0.70%, available phosphorus (P) of 2.20 cmol kg^{-1}, cation exchange capacity (CEC) of 11.74 cmol kg^{-1}, and a sandy loam texture.

Table 1. List of ethidium bromide (EtBr)-derived genotypes of cowpea at the M7 generation.

S/N	Genotype code	Treatment concentration (v/v)	Duration of treatment in hours
1	G1	0.0% (control)	0
2	G2	0.2%	2
3	G3	0.2%	4
4	G4	0.2%	8
5	G5	0.2%	16
6	G6	0.2%	32
7	G7	0.5%	2
8	G8	0.5%	4
9	G9	0.5%	8

2.3 Experimental design

The study employed a Randomized Complete Block Design (RCBD) with three replications, each measuring 4.5 m in length and 4.5 m in breadth, covering an area of 20.25 m² per replicate and a total field area of 60.75 m². Irrigation commenced on the day of seed sowing, with each plot receiving 40 liters of water daily until the plants were four weeks old. At this point, treatments were introduced. The treatments comprised drought stress, achieved by irrigating each plot with 18 mm of water twice per week, and a control condition with daily irrigation at the same rate, following the methods of Sánchez-Reinoso et al. (2019) and Badu-Appraku et al. (2021), with a few additional modifications. These treatments continued from February 19th until early April, when regular rainfall began.

Each plot was sown with three seeds per hill in nine hills per plot for each genotype and treatment, and then thinned to one uniform plant per hill two weeks after emergence. This resulted in each plot having nine plants (three plants per row) with an intra- and inter-row spacing of 50 cm by 50 cm and a border of 25 cm, totaling a plot size of 1.5 m^2.

2.3 Data collection

Morphological and yield-related traits of cowpea (*Vigna unguiculata* L. Walp.) were recorded to assess plant performance. Five weeks after sowing, observations were taken from five randomly selected plants per replication. Plant height was measured from the base at the soil surface to the tip of the main stem using a meter ruler. The number of main branches per plant was counted manually by recording all primary branches emerging from the main stem, and the number of fully expanded trifoliate leaves was also noted. The terminal leaflet of the most recently fully expanded leaf was used to measure leaflet length (from base to tip) and width (at the widest point) using a digital caliper. Peduncle length was measured from the node to the base of the inflorescence using a ruler. At maturity, yield-related traits were recorded, including the number of pods

per plant (counted manually), pod length (measured for 10 randomly selected mature pods per plant using a ruler), and number of seeds per pod (averaged from 10 pods per plant). The total number of seeds per plant was determined by manually threshing all harvested pods, and seed yield per plant was obtained by sun-drying the seeds and weighing them with a digital balance. Additionally, the 100-seed weight was measured using a precision electronic scale. The number of days to first flowering was also recorded by counting the number of days from sowing to the appearance of the first flower on each plant..

2.4 Statistical analysis

Data were analyzed through analysis of variance (ANOVA) using the Generalized Linear Model (GLM) procedure in SPSS version 20. Mean differences were determined using Duncan's Multiple Range Test (DMRT) at a significance level of $P \leq 0.05$.

2.4.1. Drought Tolerance Indices (DTIs) for each genotype

Ten drought tolerance indices were adopted based on the yield per plant under control (Yp) and drought stress (Ys) treatments, following Ajayi (2024):

The Intensity of Drought Stress (IDR), proposed by Ajayi (2024), measures the relative impact of drought stress. It is calculated by following Equation 1.

$$IDR = \frac{Ys/Yp}{\bar{Y}s} \quad (1)$$

where Ys and Yp are the yield of a genotype under drought stress and control conditions, respectively, and $\bar{Y}s$ is the mean yield of all genotypes under stress. A higher IDR indicates better drought tolerance.

The Geometric Mean Productivity (GMP) evaluates genotype performance under varying stress levels. It is calculated by following Equation 2 (Kristin et al., 1997).

$$GMP = \sqrt{Yp \times Ys} \quad (2)$$

The Harmonic Mean (HM), also proposed by Kristin et al. (1997), integrates yield performance under irrigated and drought conditions. It is calculated by following Equation 3.

$$HM = \frac{2(Yp \times Ys)}{(Yp + Ys)} \quad (3)$$

Mean Productivity (MP) represents the average yield under both control and stress conditions. It is calculated by following Equation 4 (Rosielle and Hamblin, 1981).

$$MP = \frac{Yp + Ys}{2} \quad (4)$$

The Stress Tolerance Index (STI) combines yield under stress and non-stress conditions, providing a comprehensive drought tolerance measure (Fernandez, 1992). It is calculated by following equation 5.

$$STI \frac{(Ys)(Yp)}{\left(\bar{Y}p\right)^2} \quad (5)$$

Yield Index (YI) measures genotype yield under stress relative to the average yield of all genotypes under stress (Gavuzzi et al., 1997). It is calculated by following equation 6.

$$YI = \frac{Ys}{\bar{Y}s} \quad (6)$$

The Yield Stability Index (YSI), calculated by following Equation 7 (Bouslama and Schapaugh, 1984), assesses yield stability under stress:

$$YSI = \frac{Ys}{Yp} \quad (7)$$

The Drought Resistance Index (DRI) assesses a genotype's ability to withstand drought, calculated by following Equation 8 (Moosavi et al., 2008):

$$DRI = Ys \times (\frac{Ys}{Yp}) / \bar{Y}p \quad (8)$$

The Modified Stress Tolerance Index for non-stress conditions (MSTI1) is calculated by following Equation 9 (Farshadfar and Sutka, 2002):

$$MST1 = K_1 \times STI \text{ where } K_1 = \frac{Yp^2}{\bar{Y}p^2} \quad (9)$$

The Modified Stress Tolerance Index for stress conditions (MSTI2) is calculated by following Equation 10 (Farshadfar and Sutka, 2002):

$$MST2 = K_2 \times STI \text{ where } K_2 = \frac{Ys^2}{\bar{Y}s^2} \quad (10)$$

2.4.2 Estimates of genetic parameters

Genetic parameters were calculated according to Ajayi (2024) with the following modifications. The error variance (VE) was estimated as the mean square error (MS_E) ($V_E = \sigma^2_E = MS_E$). Genotype × treatment variance (VGT) was calculated as the difference between the mean square of genotype × treatment interaction and the mean square error, divided by the number of replicates (r), i.e., ($V_{GT} = \sigma^2_{GT} = (MS_{GT} - MS_E)/r$). Genotypic variance (VG) was determined as the difference between the mean square of genotype and the mean square error, divided by the product of the number of replicates and treatments (rT), i.e., ($V_G = \sigma^2_G = (MS_G - MS_E)/rT$). Phenotypic variance (VP) was estimated as the sum of genotypic variance, genotype × treatment variance divided by the number of treatments, and error variance divided by the product of replicates and treatments, i.e., ($V_P = V_G + V_{GT}/T + V_E/rT$). The genotypic coefficient of variation (GCV) was computed as the square root of the genotypic variance divided by the grand mean ($\bar{X}$), multiplied by 100 ($GCV = \frac{\sqrt{V_G}}{\bar{X}} \times 100$). Similarly, the phenotypic coefficient of variation (PCV) was derived as the square root of the phenotypic variance divided by the grand mean ($\bar{X}$), multiplied by 100 ($PCV = \frac{\sqrt{V_P}}{\bar{X}} \times 100$). Broad-sense heritability (H^2) was calculated as the ratio of genotypic variance to the total phenotypic variance, i.e., ($H^2 = V_G/(V_G + (V_{GT}/T) + (V_E/rT))$). Genetic advance (GA) was estimated as the product of the genotypic variance divided by the square root of phenotypic variance and the selection differential (k = 2.06) ($GA = \frac{VG}{\sqrt{VP}} \times k$). Genetic advance as a percentage of the mean (GAM) was computed as the ratio of genetic advance to the grand mean, multiplied by 100 ($GAM = \frac{GA}{\bar{X}} \times 100$). Genetic parameters were categorized as cited in Ajayi et al. (2014).

Correlation analysis was conducted using SPSS version 20, with significance determined based on Pearson correlation coefficients at $P \leq 0.05$ and $P \leq 0.01$. The correlation heatmap was generated using R version 4.4.3.

3. Results

3.1 Mean performance of genotypes across control and drought stress

Tables 2 to 4 present the mean performance of various quantitative traits for different genotypes of EtBr-derived cowpea at the M7 generation under control and drought stress conditions.

Table 2. Mean performance of quantitative traits of EtBr-derived genotypes of cowpea evaluated under differential drought stress at the M7 generation

Genotype/ Trait	PH (cm)		NL		NMB		TLL (cm)		TLW (cm)		DFF		PEL (cm)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
G1	15.50±0.28^{abc}	7.53±0.29^{a}	13.00±0.58^{bcd}	7.67±0.33^{cd}	4.00±0,00^{ab}	2.67±0.33^{ab}	9.00±0.12^{cd}	4.03±1.41^{abc}	3.30±0.30^{a}	2.30±0.35^{abc}	38.75±0.24^{ab}	45.69±0.13^{d}	43.32±0.66^{e}	27.33±4.04^{cd}
G2	15.16±0.94^{ab}	7.00±0.58^{a}	15.33±0.33^{d}	5.00±0.58^{a}	5.00±0.00^{c}	1.67±0.33^{a}	6.93±0.07^{a-d}	5.00±0.58^{bcd}	5.23±0.15^{cd}	2.73±0.27^{cd}	42.78±31.37^{c}	44.58±0.24^{cd}	31.43±4.19^{d}	40.83±0.44^{e}
G3	22.73±2.34^{d}	11.60±0.35^{d}	14.33±0.33^{cd}	8.00±1.15^{cd}	3.67±0.00^{ab}	3.00±0.58^{ab}	7.63±0.23^{a-d}	6.40±0.29^{d}	5.27±017^{cd}	3.03±0.03^{d}	37.36±2.11.11^{a}	43.06±1.47^{bc}	20.06±0.88^{ab}	27.23±2.77^{cd}
G4	19.83±3.17^{bcd}	11.27±0.07^{d}	20.67±0.33^{e}	8.00±0.58^{cd}	4.00±0.00^{ab}	4.00±0.58^{bc}	9.63±0.03^{d}	2.83±0.12^{ab}	5.07±0.07^{bc}	2.03±0.03^{a}	41.67±2.20^{bc}	42.92±0.24^{bc}	27.70±5.48^{bcd}	28.00±0.58^{cd}
G5	14.00±0.87^{a}	7.00±0.58^{a}	11.00±1.15^{b}	8.67±0.33^{d}	3.50±0.29^{a}	2.67±0.33^{ab}	9.25±0.14^{cd}	4.13±0.98^{abc}	5.70±0.46^{cd}	2.83±0.17^{cd}	44.79±0.84^{cd}	45.69±0.14^{d}	18.82±0.29^{a}	30.07±0.23^{d}
G6	16.87±1.05^{abc}	9.00±0.58^{bc}	10.33±0.88^{b}	7.00±0.58^{bcd}	4.00±0.00^{ab}	3.00±0.58^{ab}	5.57±0.85^{ab}	3.53±0.75^{ab}	5.13±0.03^{cd}	2.53±0.29^{a-d}	46.11±0.28^{d}	45.28±0.28^{cd}	23.08±0.30^{abc}	22.67±2.24^{bc}
G7	20.90±1.73^{cd}	9.33±0.33^{c}	14.33±0.88^{cd}	7.67±0.33^{cd}	3.67±0.33^{ab}	3.00±0.58^{ab}	6.63±2.00^{abc}	6.10±0.15^{cd}	5.40±0.15^{cd}	3.00±0.00^{d}	55.69±0.14^{f}	43.33±1.44^{bcd}	29.62±0.69^{cd}	18.40±1.80^{ab}
G8	17.83±1.45^{a-d}	8.07±0.67^{ab}	12.00±2.08^{bc}	6.33±0.33^{abc}	4.33±0.33^{bc}	4.67±0.33^{c}	8.27±1.22^{bcd}	2.50±0.26^{a}	4.40±0.21^{b}	2.10±0.10^{ab}	44.58±0.24^{cd}	39.17±0.96^{a}	26.19±0.51^{a-d}	13.90±0.78^{a}
G9	19.80±0.75^{bcd}	8.07±0.67^{ab}	7.00±0.58^{a}	5.67±0.33^{ab}	5.00±0.00^{c}	2.33±0.33^{a}	5.27±0.43^{a}	3.43±0.64^{ab}	5.87±0.21^{d}	2.63±0.89^{a-d}	50.21±0.12^{e}	41.39±0.28^{ab}	21.25±0.29^{ab}	20.33±2.94^{ab}
Mean	18.07±0.71	8.76±0.33	13.11±0.75	7.11±0.27	4.13±0.12	3.00±0.21	7.57±0.38	4.22±0.32	5.04±0.16	2.58±0.09	44.66±1.08	43.46±0.46	26.83±1.54	25.42±1.56

Note: PH-Plant height, NL-Number of leaves per plant, NMB-Number of main branches per plant, TLL-Terminal leaflet length, TLW-Terminal leaflet width, DFF-Number of days to first flowering, PEL-Peduncle length; Values are presented as mean ± standard deviation; Different lowercase superscript letters within a column indicate significant differences among genotypes at $p \leq 0.05$ (as determined by post-hoc test).

Table 3. Mean performance of quantitative traits of EtBr-derived genotypes of cowpea evaluated under differential drought stress at the M7 generation.

Genotype/ Trait	PEPL		PPL		SPP		PL (cm)		SPPL	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
G1	47.33±0.33^{d}	47.33±6.74^{d}	33.00±0.58^{b}	125.67±33.78^{c}	11.00±0.58^{ab}	14.67±0.88^{bc}	16.10±0.59^{bc}	16.27±0.14^{c}	395.33±14.44^{bc}	1901.67±598.45^{c}
G2	41.67±1.67^{c}	79.00±0.58^{e}	46.00±1.00^{c}	95.00±0.58^{bc}	15.87±1.44^{d}	16.00±0.58^{c}	18.60±0.61^{c}	19.20±0.76^{d}	732.73±80.78^{d}	1520.33±60.31^{c}
G3	44.33±0.33^{c}	41.67±4.41^{cd}	46.00±2.52^{c}	35.33±10.33^{a}	14.67±0.67^{cd}	14.00±1.15^{b}	18.30±0.60^{c}	16.00±0.17^{c}	671.33±8.67^{d}	478.67±110.04^{a}
G4	52.00±2.00^{e}	86.00±0.58^{e}	44.33±1.67^{c}	41.00±0.58^{a}	11.33±1.67^{ab}	11.00±0.58^{a}	16.67±0.33^{bc}	15.20±0.76±^{c}	499.67±68.45^{c}	451.33±27.09^{a}
G5	19.00±0.58^{a}	27.67±0.31^{ab}	33.00±1.73^{b}	27.67±1.45^{a}	11.00±0.57^{ab}	14.00±0.00^{b}	14.90±0.35^{ab}	8.67±0.33^{a}	365.00±38.12^{bc}	387.33±20.34^{a}
G6	32.67±0.67^{b}	38.33±4.41^{cd}	19.33±2.33^{a}	36.67±8.82^{a}	13.00±0.00^{bc}	14.33±0.33^{bc}	16.90±0.91^{bc}	16.33±0.44^{c}	251.33±30.33^{ab}	530.00±136.50^{a}
G7	21.00±0.58^{a}	25.00±0.58^{a}	21.00±6.35^{a}	25.33±3.18^{a}	13.33±0.33^{bcd}	15.00±0.00^{bc}	16.63±0.47^{bc}	16.10±0.10^{c}	276.33±79.69^{ab}	380.00±47.69^{a}
G8	30.00±0.58^{b}	35.67±0.33^{bc}	16.67±2.40^{a}	47.67±1.45^{a}	10.00±0.00^{a}	14.00±0.00^{b}	17.00±1.73^{bc}	11.83±0.44^{b}	166.67±24.04^{a}	667.33±20.34^{a}
G9	32.00±0.58^{b}	36.67±1.67^{bc}	13.50±0.86^{a}	64.00±4.58^{ab}	12.50±0.29^{abc}	14.33±0.33^{bc}	12.77±1.45^{a}	10.33±1.86^{b}	169.25±14.72^{a}	918.33±74.52^{ab}
Mean	35.56±2.15	46.37±4.09	30.65±2.56	55.37±7.16	12.52±0.42	14.15±0.29	16.43±0.39	14.44±0.66	391.96±40.41	803.89±117.16

Note: PEPL-Number of peduncles per plant, PPL-Number of pods per plant, SPP-Number of seeds per pod, PL-Pod length, SPPL-Number of seeds per plant; Values are presented as mean ± standard deviation; Different lowercase superscript letters within a column indicate significant differences among genotypes at $p \leq 0.05$ (as determined by post-hoc test).

Across most traits, genotypes exhibited higher mean values under control conditions compared to drought stress. Specifically, plant height ranged from 14.00 cm (G5) to 22.73 cm (G3) under control, and from 7.00 cm (G2, G5) to 11.60 cm (G3) under drought stress. The number of leaves per plant varied between 7.00 (G9) and 20.67 (G4) under control, and from 5.00 (G2, G9) to 8.67 (G5) under stress. The number of peduncles per plant ranged from 19.00 (G5) to 52.00 (G4) under control, increasing to between 25.00 (G7) and 86.00 (G4) under stress.

Pod length varied from 12.77 cm (G9) to 18.60 cm (G2) under control, and from 8.67 cm (G5) to 19.20 cm (G2) under stress. The number of seeds per plant ranged from 10.00 (G8) to 15.87 (G2) under control, and from 11.00 (G4) to 16.00 (G2) under drought. Seeds per pod varied widely, from 166.67 (G8) to 732.73 (G2) under control, and from 380.00 (G7) to 1901.67 (G1) under stress.

The 100-seed weight ranged between 12.17 g (G1) and 16.57 g (G2) under control, and from 6.23 g (G7, G9) to 19.17 g (G4) under stress. Seed yield per plant ranged from 22.13 g (G8) to 122.24 g (G2) under control, and from 23.23 g (G7) to 342.01 g (G1) under drought stress. Notably, genotypes G1 and G2 demonstrated superior resilience to drought stress across multiple traits.

3.2 Percentage change in quantitative traits

The percentage reduction in quantitative traits among EtBr-derived genotypes of cowpea evaluated under differential drought stress conditions at the M7 generation is presented in Table 5.

Table 4. Mean performance of quantitative traits of EtBr-derived genotypes of cowpea evaluated under differential drought stress at the M7 generation.

Genotype/Traits	SW (g)		SYPL (g)	
	Control	Stress	Control	Stress
G1	12.17±0.60^{a}	17.97±0.03^{c}	48.27±4.06^{ab}	342.01±107.97^{b}
G2	16.57±0.64^{d}	18.47±0.29^{c}	122.24±17.22^{d}	280.85±12.88^{b}
G3	15.60±0.61^{cd}	17.83±0.12^{c}	104.78±4.99^{cd}	85.28±19.58^{a}
G4	14.87±1.51^{bcd}	19.17±0.17^{c}	76.06±17.14^{bc}	86.42±4.52^{a}
G5	13.00±0.58^{ab}	12.73±0.27^{b}	47.89±7.08^{ab}	49.42±3.52^{a}
G6	12.57±0.23^{a}	11.73±3.45^{b}	31.55±3.67^{a}	54.46±9.03^{a}
G7	14.00±0.29^{abc}	6.23±0.50^{a}	39.15±11.96^{a}	23.23±1.20^{a}
G8	13.27±0.37^{ab}	13.67±0.60^{b}	22.13±3.27^{a}	90.98±1.90^{a}
G9	14.95±0.59^{bcd}	6.23±0.37^{a}	25.46±3.13^{a}	56.94±4.25^{a}
Mean	14.11±0.34	13.78±0.99	57.50±7.15	118.84±23.17

Note: SW-Seed weight, SYPL-Seed yield per plant; Values are presented as mean ± standard deviation; Different lowercase superscript letters within a column indicate significant differences among genotypes at $p \leq 0.05$ (as determined by post-hoc test).

The highest reductions were observed in plant height (59.24%) and 100-seed weight (58.33%) in genotype G9. Genotype G2 exhibited the highest reduction in the number of leaves (67.38%) and the number of main branches (66.60%). Genotype G4 recorded the highest reductions in terminal leaflet length (70.61%), terminal leaflet width (59.96%), and the number of peduncles per plant (19.87%). Genotype G7 showed the highest reduction in days to first flowering (22.20%) and seed yield per plant (40.66%), while the greatest reduction in peduncle length (46.93%) was observed in genotype G8. Genotype G3 had the highest reductions in the number of pods per plant (23.20%), the number of seeds per pod (4.57%), and the number of seeds per plant (28.70%). The highest reduction in pod length (41.81%) was observed in genotype G5.

3.3 Drought Tolerance Indices

Table 6 summarizes the drought tolerance indices (DTIs) for the EtBr-derived cowpea genotypes under control and drought stress conditions. Genotypes G1 and G2 exhibited superior drought tolerance with high values in multiple indices, including GMP, STI, and DRI. In contrast, genotypes G5 and G7 demonstrated the lowest drought tolerance, with the lowest GMP and STI values among the evaluated genotypes. Table 7 ranks the genotypes based on their drought tolerance indices in response to differential drought stress at the M_7 generation. Genotypes G1 and G2 showed high tolerance to drought with rank mean (RM) values less than 4.0. Genotypes G3, G4, and G8 displayed moderate tolerance with RM values between 4.0 and 4.7. The remaining genotypes, G5, G6, G7, and G9, are highly susceptible to drought stress, with RM values exceeding the grand mean of 4.93.

3.4 Estimates of genetic parameters

Table 8 presents the genetic parameters of quantitative traits for EtBr-derived cowpea genotypes evaluated under differential drought stress at the M7 generation. Traits such as plant height, terminal leaflet width, number of peduncles per plant, seeds per pod, pod length, and seed yield per plant showed high broad-sense heritability of 84.41%, 70.59%, 82.34%, 70.25%, 80.20%, and 60.08%, respectively, indicating that genetic factors strongly influenced these traits. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were generally high for most traits, with seed yield per plant exhibiting the highest GCV of 71.54% and PCV of 92.29%. Most traits exhibited moderate to high genetic advance as a percentage of the mean (GAM), except for the number of days to the first flowering, suggesting significant potential for improvement through selective breeding. These parameters highlight the traits' potential for genetic improvement and the effectiveness of EtBr treatment in inducing beneficial genetic variations.

3.5 Heatmap for Pearson's correlation analysis

Figure 1 displays a heatmap of Pearson's correlation coefficients showing the relationships between various drought tolerance indices (DTIs) and quantitative traits of EtBr-derived cowpea genotypes evaluated at the M7 generation under drought stress.

Several commonly used DTIs—GMP, HM, MP, and STI, demonstrated strong, significant positive correlations ($r \geq 0.70$)

Table 5. Percentage reduction of the quantitative traits of EtBr-derived genotypes of cowpea evaluated under differential drought stress at the M7 generation.

Genotype	PH (cm)	NL	NMB	TLL (cm)	TLW (cm)	DFF	PEL (cm)	PEPL	PPL	SPP	PL (cm)	SPPL	SW (g)	SYPL (g)
G1	51.42	41.00	33.25	55.22	30.30	-17.92	36.91	0.00	-249.08	-33.36	-1.06	-381.03	-47.66	-608.54
G2	53.83	67.38	66.60	27.85	47.80	-4.22	-29.91	-89.58	-106.52	-0.82	-3.23	-107.49	-11.47	-129.75
G3	48.97	44.17	18.26	16.12	42.50	-15.23	-35.74	6.00	23.20	4.57	12.57	28.70	-14.29	18.61
G4	43.17	61.30	0.00	70.61	59.96	-3.00	-1.08	19.87	7.51	2.91	8.82	9.67	-28.92	-13.62
G5	50.00	21.18	23.71	55.35	50.35	-2.02	-59.78	-119.32	16.15	-27.27	41.81	-6.12	2.08	-3.19
G6	46.65	32.24	25.00	36.62	50.68	1.81	1.78	-27.55	-89.71	-10.23	3.37	-110.88	6.68	-72.61
G7	55.36	46.48	18.26	7.99	44.44	22.20	37.88	-98.43	-20.62	-12.53	3.19	-37.52	55.5	40.66
G8	54.74	47.25	-7.85	69.77	52.27	12.15	46.93	-38.90	-185.96	-40.00	30.41	-300.39	-3.01	-311.12
G9	59.24	19.00	53.40	34.91	55.20	17.57	4.33	-30.22	-374.07	-14.64	19.11	-442.59	58.33	-123.64

Note: PH-Plant height, NL-Number of leaves per plant, NMB-Number of main branches per plant, TLL-Terminal leaflet length, TLW-Terminal leaflet width, DFF-Number of days to first flowering, PEL-Peduncle length, PEPL-Number of peduncles per plant, PPL-Number of pods per plant, SPP-Number of seeds per pod, PL-Pod length, SPPL-Number of seeds per plant, SW-Seed weight, SYPL-Seed yield per plant; Negative signs indicate a percentage increase for the trait under consideration for specific genotypes.

Table 6. Drought tolerance indices (DTIs) of EtBr-derived genotypes of cowpea evaluated under differential drought stress at the M7 generation.

Genotype	IDR	GMP	HM	MP	STI	YI	YSI	DRI	MST1	MST2
G1	0.060	128.487	84.600	195.140	4.993	2.878	7.085	42.144	0.705	8.282
G2	0.019	185.287	170.340	201.545	10.384	2.363	2.298	11.222	4.520	5.585
G3	0.007	94.529	94.030	95.030	2.703	0.718	0.814	1.207	3.321	0.515
G4	0.010	81.075	80.910	81.240	1.988	0.727	1.136	1.708	1.750	0.529
G5	0.009	48.649	48.643	48.655	0.716	0.416	1.032	0.887	0.694	0.173
G6	0.015	41.451	39.954	43.005	0.520	0.458	1.726	1.635	0.301	0.210
G7	0.008	23.230	23.230	23.230	0.163	0.195	1.000	0.404	0.163	0.038
G8	0.035	44.871	35.601	56.555	0.609	0.766	4.111	6.505	0.148	0.586
G9	0.019	38.075	35.187	41.200	0.438	0.479	2.236	2.215	0.196	0.230
Grand mean	0.017	82.664	77.501	88.170	2.067	1.000	2.067	4.272	1.000	1.000

Note: IDR-Intensity of drought resistance, GMP-Geometric mean productivity, HM-Harmonic mean, MP-Mean productivity, STI-Stress tolerance index, YI-Yield index, YSI-Yield stability index, DRI-Drought resistance index, MST1-Modified stress tolerance index for the non-stress conditions, MST2-Modified stress tolerance index for stress conditions.

Table 7. Ranking of EtBr-derived genotypes of cowpea based on DTI in response to differential drought stress at the M7 generation.

Genotype	IDR	GMP	HM	MP	STI	YI	YSI	DRI	MST_1	MST_2	RS	RM	STDR
G1	1	2	3	2	2	1	1	1	4	1	18	1.80	1.03
G2	3	1	1	1	1	2	3	2	1	2	17	1.70	0.82
G3	8	3	2	3	3	5	9	7	2	5	47	4.70	2.54
G4	5	4	4	4	4	4	6	5	3	4	43	4.30	0.82
G5	6	5	5	6	5	8	7	8	5	8	63	6.30	1.34
G6	4	7	6	7	7	7	5	6	6	7	62	6.20	1.03
G7	7	9	9	9	9	9	8	9	8	9	86	8.60	0.70
G8	2	6	7	5	6	3	2	3	9	3	46	4.60	2.37
G9	3	8	8	8	8	6	4	4	7	6	62	6.20	1.93
Grand mean											49.33	4.93	1.39

Note: IDR-Intensity of drought resistance, GMP-Geometric mean productivity, HM-Harmonic mean, MP-Mean productivity, STI-Stress tolerance index, YI-Yield index, YSI-Yield stability index, DRI-Drought resistance index, MST1-Modified stress tolerance index for the non-stress conditions, MST2-Modified stress tolerance index for stress conditions, RS-Rank sum, RM-Rank mean, STD-Standard deviation of rank; Ranks 1–5 = Tolerance, 6–9 = Susceptibility, RM < Grand mean and not up to 4.0 = High tolerance, RM very close to Grand mean = moderate tolerance; RM > Grand mean = Highly susceptible.

Table 8. Estimates of genetic parameters of quantitative traits of EtBr-derived genotypes of cowpea evaluated under differential drought stress at the M7 generation.

Trait	Grand mean	V_G	V_P	V_{GT}	V_E	GCV (%)	PCV (%)	H^2 (%)	GAM (%)
PH	13.42	4.33	5.13	0.30	3.89	15.51	16.88	84.41	29.44
NL	10.11	4.47	7.80	6.32	1.04	20.91	27.64	57.31	32.64
NMB	3.57	0.14	0.48	0.59	0.32	10.48	19.41	29.17	11.76
TLL	5.89	0.58	1.89	2.00	1.85	12.93	23.34	30.69	14.77
TLW	3.81	0.24	0.34	0.15	0.17	12.86	15.30	70.59	22.31
DFF	44.06	7.44	17.87	19.98	2.66	41.09	63.69	41.63	8.22
PEL	26.13	32.37	56.80	44.33	13.64	21.77	28.84	56.99	33.83
PEPL	40.97	244.95	297.49	100.15	14.82	38.20	42.09	82.34	71.39
PPL	43.01	353.49	632.37	487.90	209.59	43.71	58.47	56.71	67.31
SPP	13.34	1.70	2.42	0.85	1.79	9.77	11.66	70.25	16.79
PL	15.44	5.59	6.97	2.08	2.03	15.31	17.10	80.20	28.24
SPPL	597.93	89536.28	161484.50	123309.10	61762.05	50.04	67.21	55.45	76.76
SW	13.95	7.26	13.41	11.33	2.96	19.31	26.25	54.14	29.32
SYPL	88.17	3978.53	6621.65	4632.05	1962.55	71.54	92.29	60.08	114.23

Note: V_G-Genotypic variance, V_P-Phenotypic variance, V_{GT}-Genotype × treatment variance, V_E-Error variance, GCV-Genotypic coefficient of variation, PCV-Phenotypic coefficient of variation, H^2-Broad-sense heritability, GAM-Genetic advance as percent of the mean, PH-Plant height, NL-Number of leaves per plant, NMB-Number of main branches per plant, TLL-Terminal leaflet length, TLW-Terminal leaflet width, DFF-Number of days to first flowering, PEL-Peduncle length, PEPL-Number of peduncles per plant, PPL-Number of pods per plant, SPP-Number of seeds per pod, PL-Pod length, SPPL-Number of seeds per plant, SW-Seed weight, SYPL-Seed yield per plant.

with key yield-related traits, especially peduncle length, seed yield per plant, number of pods per plant, and number of seeds per plant. These relationships highlight the reliability of these indices in identifying genotypes capable of maintaining high productivity under both drought and optimal conditions. The YI also showed significant positive correlations ($r \geq 0.70$) with seed yield per plant, the number of pods per plant, and seeds per plant, underscoring its effectiveness in identifying genotypes that sustain productivity under drought stress compared to non-stress environments. Likewise, DRI correlated strongly with the same yield-related traits (seed yield per plant, number of pods per plant, and the number of seeds per plant), reinforcing its usefulness as a measure of drought resilience in cowpea. The YSI showed significant correlations at or above the 0.70 threshold with seed yield per plant and pods per plant, indicating its strength in selecting genotypes that maintain yield stability across different environments, including water-limited conditions. The IDR also showed significant positive correlations ($r \geq 0.70$) with seed yield per plant, pods per plant, and seeds per plant. Adding to these, the MST1 and MST2 both showed significant positive correlations ($r \geq 0.7$) with the primary yield traits—seed yield per plant, number of pods per plant, and number of seeds per plant. MST1 is more relevant under non-stress conditions, while MST2 specifically addresses stress conditions; nonetheless, both indices effectively identify genotypes that deliver stable seed yield under their respective regimes.

Importantly, none of the DTIs exhibited significant positive correlations ($r \geq 0.70$) with vegetative growth traits such as plant height, number of main branches per plant, or number of leaves per plant. This trend suggests that DTIs are more strongly associated with reproductive output rather than vegetative biomass under drought conditions. In fact, most vegetative traits showed weak or even insignificant negative correlations with several DTIs, implying that these traits may contribute little—or potentially detract—from drought tolerance. These findings highlight the need for breeding programs to prioritize yield-related traits such as number of pods per plant, peduncle length, seed yield per plant, and the number of seeds per plant as key selection criteria for enhancing drought tolerance and overall productivity in cowpea.

4. Discussion

Understanding the range of genetic variability induced by mutagenesis and pinpointing drought-tolerant genotypes is essential for improving yield in drought-prone regions of SSA. In this study, drought stress significantly affected most traits, except peduncle length and 100-seed weight, highlighting its adverse impact. Genetic variability influenced all traits, revealing notable differences among the cowpea genotypes. Non-significant replicate effects confirmed consistent experimental conditions. Significant T × G interactions across all traits indicated varying genotype responses to drought, which is crucial for selecting drought-tolerant genotypes. These findings underscore the importance of selecting and breeding drought-resilient cowpea genotypes due to the significant genetic variability and differential responses observed (Yahaya et al., 2019; Badu-Apraku et al., 2021).

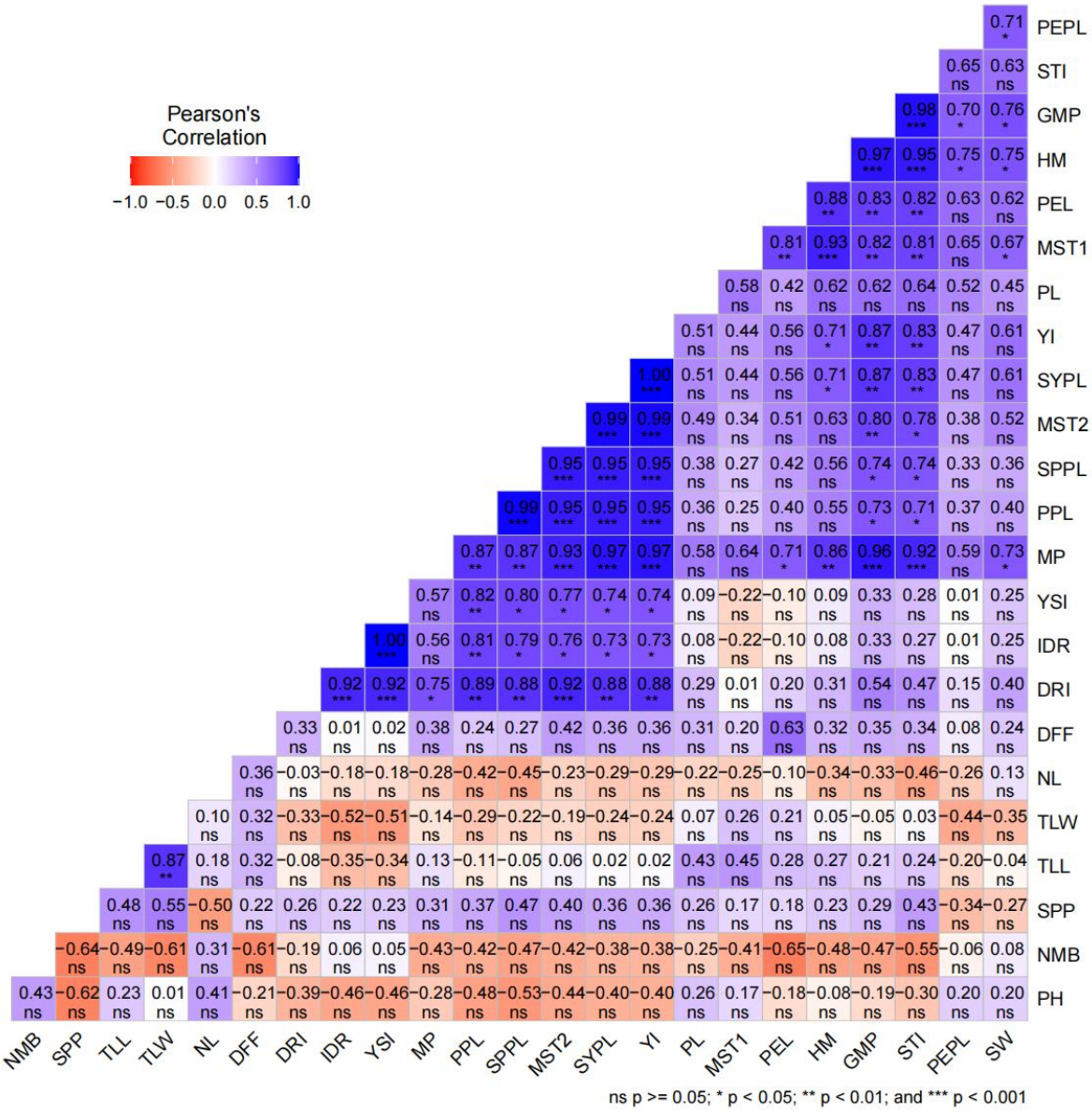

Figure 1. Heatmap showing Pearson's correlation between drought tolerance indices (DTIs) and quantitative traits of *EtBr*-derived cowpea genotypes at the M7 generation under drought stress. IDR-Intensity of drought resistance, GMP-Geometric mean productivity, HM-Harmonic mean, MP-Mean productivity, STI-Stress tolerance index, YI-Yield index, YSI-Yield stability index, DRI-Drought resistance index, MST1-Modified stress tolerance index for the non-stress conditions, MST2-Modified stress tolerance index for stress conditions, PH-Plant height, NL-Number of leaves per plant, NMB-Number of main branches per plant, TLL-Terminal leaflet length, TLW-Terminal leaflet width, DFF-Number of days to first flowering, PEL-Peduncle length, PEPL-Number of peduncles per plant, PPL-Number of pods per plant, SPP-Number of seeds per pod, PL-Pod length, SPPL-Number of seeds per plant, SW-Seed weight, SYPL-Seed yield per plant.

These findings highlight the necessity of choosing and developing drought-resistant cowpea genotypes because of the considerable genetic heterogeneity and varied responses observed. Several studies with cowpea genotypes and other crop species

have reported sufficient levels of variability among genotypes under varied drought stress (Ajayi, 2020; Santos et al., 2020; Lao et al., 2022; Elbath et al., 2023; Karami et al., 2024).

None of the genotypes consistently performed above average for all vegetative traits across control and stress conditions. Each genotype had specific traits where it performed above average, but no single genotype excelled in all traits. Similar inconsistencies in performance under differential drought stress have been reported in many crop plants, especially among yield and its contributing traits (Ajayi, 2020; Ahmed et al., 2023; Sanogo et al., 2023). However, genotype G2 demonstrated the best overall performance under control conditions, excelling in most yield traits compared to G1 and G4. Under stress conditions, G4 showed superior resilience and outperformed the others in key traits like the number of peduncles per plant and 100-seed weight; however, G2 outperformed it for traits such as the number of seeds per pod, pod length, number of seeds per plant, and seed yield per plant.

G1 demonstrated the highest performance under stress in terms of seed yield per plant and the number of seeds per plant, but it is generally surpassed by G2 and G4 in other traits. It also exhibited similar values under both control and drought stress conditions. To explain why G1 displayed comparable values for the number of peduncles under both control and drought stress, several factors may be involved, including drought tolerance, trait stability, and biological trade-offs. First, G1 may possess inherent traits, such as deep rooting or effective osmotic adjustment, which enable it to sustain peduncle production under water-limited conditions, indicating true drought tolerance. This contrasts with genotypes like G2 and G4, which exhibit increased peduncle numbers under drought stress, suggesting a compensatory response rather than inherent tolerance. Second, peduncle development in G1 may be less sensitive to soil moisture fluctuations than traits like pods per plant or seeds per pod, which show more pronounced stress-induced variation, indicating peduncle trait stability. Third, the statistical data show no significant difference in peduncle number between control and drought conditions in G1, as the means are similar and the standard errors overlap. Lastly, from a biological trade-off perspective, G1's consistent peduncle production under both conditions suggests a stable reproductive strategy, which is valuable for breeding drought-resilient cowpea varieties. The performance of G2 and G4 suggests that these genotypes are strong candidates for breeding programs aimed at improving cowpea yields. G2 is ideal for breeding efforts focused on maximizing yield under both normal and stress conditions, given its superior performance in most traits. Meanwhile, G4's resilience under drought stress makes it valuable for developing drought-tolerant varieties, ensuring stable yields in challenging environments.

Morphological traits are key determinants of yield in cowpea, and in this study, drought stress significantly reduced these traits in most genotypes. The least affected traits were the number of seeds per plant and seed yield per plant, which increased by approximately 150% and 134%, respectively. In contrast, the most affected traits under drought stress were plant height and terminal leaflet width,

which decreased by 51% and 48%, respectively. This aligns with previous research showing that drought limits water absorption, slows growth, and disrupts photosynthesis, mineral uptake, protein synthesis, and carbohydrate metabolism, consequently adversely affecting the growth and yield of crop species (Santos et al., 2020; Ghanem and Al-Farouk, 2024; Ikram et al., 2024). However, after the onset of rainfall, the drought-stressed plants outperformed the control plants in yield and yield-related traits. This can be attributed to physiological and adaptive responses such as deeper root development, improved water use efficiency, osmoprotectant accumulation, and optimized photosynthesis and hormonal balance, all of which promote rapid recovery and enhanced productivity (Mwale et al., 2017; Santos et al., 2020). These mechanisms of drought tolerance have been categorized into various classes based on several studies (Hall, 2012; Beebe et al., 2013).

The genotypes G1, G2, G3, G5, G6, G7, and G9 exhibited reductions across all vegetative traits (plant height, number of leaves, number of main branches, terminal leaflet length, and width) under drought stress. Notably, days to first flowering and peduncle length tended to increase under these conditions. Despite these reductions, G1 and G2 consistently showed increased yield traits under drought, indicating that they possess genetic characteristics conferring resilience and productivity under water-limited conditions. These genotypes were specifically more resilient for early flowering, number of seeds per pod, pod length, and number of seeds per plant, with the least reduction in terminal leaflet width. This makes G1 and G2 promising candidates for breeding programs focused on enhancing drought tolerance and yield stability in cowpeas, potentially contributing to improved food security and agricultural sustainability in drought-prone regions. Drought stress delayed flowering in five genotypes (G1–G5) by 2 to 18 percent, with G1 showing the longest delay, while only G3 experienced yield reduction under these conditions. In contrast, flowering was accelerated in genotypes G6–G9, with increased yield in all but G7. Seed weight also increased under drought stress in G1 to G4 and G8 by 3 to 48 percent (highest in G1). The observed increases in seed yield, ranging from 3 percent in G5 to 608 percent in G1, were largely due to increases in the number of pods per plant, seeds per pod, and seed weight per plant, in contrast to the findings of Batieno *et al.* (2016). This diverse response suggests a collection of genotypes that employ both drought escape and tolerance mechanisms, consistent with Yahaya's findings (Yahaya et al., 2019) and Ikram et al. (2024).

Rankings based on DTIs are crucial for cowpea breeding programs that focus on drought tolerance (Ajayi, 2020), and it is clear that the ranking of genotypes in the present study conforms to those of Eid and Sabry (2019) and Yahaya et al. (2023). The genotypes varied widely for all the DTIs, and this wide variability provides breeders with a broad selection pool for developing drought-resistant cowpea varieties (Deffo et al., 2024). This level of variability for DTIs agrees with several studies on cowpea (Ajayi, 2020; Ajayi, 2024; Deffo et al., 2024) and other crop species (Bennani et al., 2017; Karami et

al., 2024). Breeders can study the correlation between these traits and actual drought tolerance, helping them select the best parent plants for breeding programs. Targeting these highly variable parameters can lead to significant improvements in specific traits, directly contributing to drought resistance. Additionally, the variability indicates the potential for hybrid development by crossing genotypes with complementary traits to produce offspring with heightened drought tolerance. High-tolerant genotypes like G1 and G2 with above-average values for all drought tolerance indices should be used as parent plants to introduce drought-resistant traits into new varieties. Conversely, highly susceptible genotypes such as G5, G6, G7, and G9, showing below-average values for most indices, should be improved by incorporating drought-tolerant genes. Intermediate genotypes like G3, G4, and G8 can serve as breeding targets to create a range of drought tolerance levels. This approach will enhance genetic diversity and aid in developing resilient cowpea varieties suitable for drought-prone regions. Anwar et al. (2011), Bennani et al. (2017), and Ajayi (2024) discuss the significance of various DTIs and the performance of different cowpea and wheat genotypes under stress conditions, supporting the present findings.

Genetic parameters observed under drought stress offer valuable insights for cowpea breeding programs (Ajayi, 2020), underscoring the importance of high heritability in key drought-tolerant traits (Songsri et al., 2008; Choudhary et al., 2021). In an assessment of genetic variability among 24 cowpea accessions, Ajayi (2020) found considerable variability, moderate to high heritability, and high genetic advance as a percentage of the mean (GAM) in essential yield traits, including the number of pods per plant, seeds per pod, seeds per plant, and seed yield per plant. Similar trends of high heritability were observed in maize hybrids for traits like grain yield and plant height under drought conditions across multiple field trials (Badu-Apraku et al., 2021). In this study, traits such as plant height, terminal leaflet width, peduncles per plant, pod length, and seed yield per plant, exhibiting high heritability, are primarily influenced by genetic factors and are thus well-suited for selection. The high genetic advance observed in most traits, except for the number of main branches, terminal leaflet length, days to first flowering, and number of seeds per pod, suggests strong potential for improvement through selective breeding. Notably, substantial genetic and phenotypic variation in traits like seed yield per plant indicates significant diversity, supporting the selection of superior genotypes (El-Rawy and Hassan, 2014). Furthermore, the data highlight the potential of EtBr-induced mutants to enhance drought tolerance, presenting a viable pathway for developing resilient cowpea varieties for drought-prone regions.

G7's drought sensitivity and poor performance in peduncles per plant, pods per plant, and seed yield can be attributed to its origin and mutagenesis treatment. Unlike G2—a mutant of the untreated parent G1 exposed to 0.2% ethidium bromide (EtBr) for 2 hours, which showed enhanced drought tolerance—G7 was treated with a higher EtBr concentration (0.5% for 2 hours) and exhibited the poorest drought performance among all genotypes. This contrast highlights

the dose-dependent nature of EtBr-induced mutagenesis: while lower concentrations like 0.2% in G2 likely induced beneficial mutations, the higher dose used for G7 may have caused harmful changes affecting plant vigor and stress response.

These findings emphasize that although EtBr is a potent mutagen capable of generating useful genetic variability, its effectiveness hinges on careful calibration of dose and exposure time. G2's superior drought traits confirm the potential of EtBr mutagenesis for stress tolerance improvement, while G7's performance warns against excessive mutagen levels that may impair critical physiological functions. This variability points to the importance of optimizing EtBr concentration and exposure duration to maximize beneficial outcomes. The success of G2 in surpassing G1 also emphasizes the role of genetic diversity created through mutagenesis in breeding programs. Breeders can develop more resilient cowpea varieties by selecting mutants with enhanced traits. Additionally, further research into the genetic changes induced by EtBr treatment could provide deeper insights into drought tolerance mechanisms and improve future breeding strategies (Yuliasti & Reflinur, 2015; Chaudhary et al., 2019).

Understanding the relationships among morphological traits and yield-based DTIs under drought stress is essential for plant breeders to establish effective criteria for selecting drought-tolerant varieties. The most appropriate selection indices are those that positively correlate with seed yield and yield-contributing traits under both optimal and drought-stressed conditions. Nonetheless, indices that are positively correlated with yield and its components solely under drought stress remain valuable for identifying genotypes suited to drought-prone environments. In this study, the lack of correlation between yield in stressed and control conditions (the correlation table for control is not presented) suggests that genotype selection should not focus solely on performance under optimal conditions. This finding agrees with El-Refaee et al. (2023) but contrasts with those of Sanogo et al. (2023) and Deffo et al. (2024). However, based on the correlation heatmap under drought stress conditions, correlations between DTIs and quantitative traits of cowpea were observed. Peduncle length showed strong positive correlations with MP, STI, GMP, MST2, and HM, highlighting its role in drought tolerance. The number of pods per plant was consistently correlated with DTIs like YI, MST2, DRI, IDR, YSI, and MP, indicating its importance for drought resilience. Seed yield per plant exhibited positive correlations with DTIs, including GMP, HM, STI, YI, DRI, IDR, YSI, MP, and MST2, suggesting it is beneficial for breeding drought-tolerant cowpea. Additionally, the number of seeds per plant showed positive associations with DTIs such as GMP, MP, STI, YI, MST2, DRI, IDR, and YSI under drought stress conditions, further confirming its relevance in drought tolerance.

The strong positive correlations between peduncle length and several drought tolerance indices, including MP, STI, GMP, MST2, and HM, suggest that peduncle length is more than just a morphological trait. It appears to contribute significantly to cowpea performance under drought conditions,

possibly reflecting improved reproductive development or greater yield stability. This highlights peduncle length as an important trait to include in selection strategies for drought tolerance. Likewise, the number of pods per plant showed consistent positive correlations with several DTIs such as YI, MST2, DRI, IDR, YSI, and MP, underscoring its importance in conferring drought resilience. Since the number of pods per plant directly determines potential seed output, genotypes that sustain pod production under drought are more likely to maintain stable yields.

Seed yield per plant exhibited strong positive associations with a wide range of DTIs, including GMP, HM, STI, YI, DRI, IDR, YSI, MP, and MST2, confirming its reliability as a key indicator of drought tolerance. Breeding programs that focus on maintaining or improving seed yield under drought conditions are therefore more likely to identify genotypes with strong resilience.

In addition, positive relationships between the number of seeds per plant and various DTIs further support the role of reproductive output in ensuring drought adaptation. Genotypes that can continue to produce seeds under drought are better suited for environments where water availability is limited.

Overall, these results point to the importance of reproductive traits—particularly peduncle length, number of pods per plant, number of seeds per plant, and seed yield per plant—as strong indicators of drought tolerance. Including peduncle length in selection criteria may improve the identification of genotypes with enhanced performance under stress, possibly through more efficient flowering or seed development. While vegetative traits showed weak or inconsistent associations with the DTIs, the reproductive traits demonstrated consistent and meaningful correlations. Research findings by El-Refaee et al. (2023), Sanogo et al. (2023), and Deffo et al. (2024) also confirmed the usefulness of these DTIs for their strong positive correlations with yield and yield-related traits under stressed conditions. The present findings also support those of Ajayi (2024) and Ghanem and Al-Farouk (2024), who stressed the importance of DTIs in the selection of drought-tolerant crop species.

5. Conclusion

This study emphasizes the significance of induced genetic variability in enhancing drought tolerance among cowpea genotypes. Drought stress notably reduced most vegetative and yield traits, yet genotypes such as G2 and G4 exhibited remarkable resilience, making them prime candidates for breeding programs aimed at improving cowpea productivity in drought-prone regions. Importantly, G2, developed through EtBr-induced mutagenesis, demonstrated superior performance, highlighting the effectiveness of chemical mutagenesis in enhancing drought resistance.

The study identified G2 (treated with 0.2% EtBr for 2 hours) as the most highly tolerant genotype, followed by G1 (untreated control). Genotypes G4 (0.2% EtBr for 8 hours), G3 (0.2% EtBr for 4 hours), and G8 (0.5% EtBr for 4 hours) demonstrated moderate tolerance. In contrast, G6 (0.2% EtBr for 32 hours), G7 (0.5% EtBr for 2 hours), G9 (0.5% EtBr for 8 hours), and G5 (0.2% EtBr for 16 hours)

were identified as highly susceptible to drought stress.

Genetic parameter estimates further revealed high heritability and GAM for key traits, including plant height, peduncle length, and seed yield per plant. This indicates that these traits are primarily governed by genetic factors and are well-suited for selection. For instance, the high heritability observed in seed yield per plant (60.08%) suggests significant potential for genetic improvement through selective breeding. The substantial genetic variability among genotypes, combined with high heritability estimates, provides a robust foundation for selecting drought-tolerant traits.

The positive correlations between key DTIs and yield traits such as the number of pods and seed yield per plant indicate that these parameters are crucial for breeding resilient cowpea varieties. Prioritizing these traits in breeding strategies will contribute to the development of drought-adapted genotypes, thereby enhancing crop performance and ensuring food security in drought-affected regions.

Author Contribution

Conceptualization, A.T. Ajayi; Methodology, A.T. Ajayi, O.E. Oladipo, O.O. Dada, and A.A. Amoo; Statistical analysis, A.T. Ajayi; writing–original draft preparation, M.E. Momoh; writing–review and editing, A.T. Ajayi. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors acknowledge the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, for providing the cowpea accession used in this study. Appreciation is also extended to the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria, for providing the necessary facilities for the research.

Availability of data and materials

Data will be available on a formal request from the corresponding authors.

Funding

Not applicable.

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